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Isolation of interleukin 2-induced immediate-early genes

(differential cloning/human T cells/cytokine-responsive genes)

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ABSTRACT Clonal expansion of antigen-reactive T lymphocytes is driven by the lymphokine interleukin 2 (IL-2). To further elucidate the mechanisms of IL-2 action, we have utilized a differential hybridization procedure to clone IL-2-induced immediate-early genes from an IL-2-stimulated human T-cell cDNA library. To increase the frequency of IL-2-induced transcripts represented in the library, the protein synthesis inhibitor cycloheximide was included during the 2-hr IL-2 stimulation to superinduce gene expression, and the uridine analogue 4-thiouridine was utilized to enable selective purification of newly synthesized transcripts. From the enriched library, we have isolated eight IL-2-induced genes, six of which represent previously unrecognized human sequences. Northern blot analysis revealed that the induction of seven of the genes is specific to the IL-2-mediated G₁ "progression" phase of the cell cycle, in that only one gene is also induced during the T-cell receptor-triggered G₀–G₁ "competence" phase. These results indicate that the effects of IL-2 are mediated by the specific induction of a number of immediate-early genes and provide a means with which to further delineate the mechanisms whereby IL-2 stimulates T-lymphocyte proliferation and differentiation. The methods described in this report should also be of general utility in the dissection of the signaling pathways activated by diverse cytokine receptors.

Over the past several years, seminal studies have established that T-cell activation occurs as a two-step process (1). Quiescent lymphocytes are stimulated initially by antigen-presenting cells via the T-cell antigen receptor/CD3 complex (TCR/CD3), together with accessory molecules such as CD28 (2), which enables the cells to produce and respond to interleukin 2 (IL-2) (3–6). Subsequently, the interaction of IL-2 with its receptor (IL-2R) promotes cell cycle progression and DNA replication (3, 4, 6–8). Thus, these two cell surface receptor systems trigger different cellular processes: the G₀–G₁ transition (cell cycle competence) is activated by the TCR/CD3 complex, whereas G₁ progression to S phase (cell cycle progression) is promoted by the IL-2R (7–9). Consistent with these findings, the early biochemical events triggered by these two receptor systems are distinct. TCR/CD3 stimulation activates rapid tyrosine-specific phosphorylation of several substrates (10–12), followed by phosphatidylinositol (PI) hydrolysis and subsequent elevation of intracellular free calcium and activation of the calcium- and phospholipid-dependent serine/threonine-specific protein kinase C (PKC) (13, 14). Tyrosine-specific (12, 15) and serine/threonine-specific (16) phosphorylation also occurs within minutes of IL-2R activation. However, IL-2-mediated signal transduction does not involve PI hydrolysis (17), elevations in intracellular calcium (18), or PKC activation (19, 20). Moreover, the serine/threonine-specific kinase Raf-1 is activated by the IL-2R but not by the TCR/CD3 complex (21, 22).

Since the TCR/CD3 complex and the IL-2R activate distinct early biochemical signaling pathways and effect changes in separate stages of the cell cycle, it may be assumed that some of the genes stimulated by the two receptor pathways will also be different. To date, a significant number of early genes have been shown to be expressed in response to TCR/CD3 complex activation, including several previously characterized protooncogenes and cytokines (23, 24), as well as a substantial number of previously unrecognized genes (25). Several of the cellular protooncogenes, such as *c-fos*, *c-myc*, and *c-raf-1*, are expressed after both TCR/CD3 and IL-2R activation (22, 23, 26), while induction of the *c-myc* gene is unique to IL-2R stimulation (8). To enable further analysis of the genetic program expressed during the course of lymphocyte activation, we have sought to identify additional IL-2-induced early genes.

Although it is relatively straightforward to determine whether previously characterized genes are expressed in response to receptor triggering, it is somewhat more difficult to identify new genes, given the background of the many genes already expressed constitutively. One approach that has enabled the isolation of a number of growth factor-induced genes is based on the differential hybridization of cDNA libraries, using probes obtained from activated cells compared with quiescent cells (27–29). However, this method is limited by its sensitivity, detecting only those transcripts that are expressed in relatively high abundance, at a level >500 copies per cell (27). To augment the level of gene expression above this threshold, cycloheximide (CHX) has been used, as the inhibition of protein synthesis superinduces the expression of a number of genes, especially those that have short-lived transcripts (27–29). To further enrich for newly induced genes, a thiol selection procedure has been employed whereby growth factor-induced RNA transcripts are selectively labeled with thiol-derivatized uridine, enabling subsequent purification of the labeled transcripts by phenylmercury agarose chromatography (30, 31). In this manner, the newly synthesized transcripts may be separated from the transcripts derived from housekeeping-type genes that were present prior to stimulation (30, 31). This thiol-labeling procedure was used in the generation of a cDNA library from newly synthesized RNA of human peripheral blood T cells stimulated for 2 hr with IL-2 and CHX. By differential screening of this library, eight IL-2-induced genes have been isolated, six of which are newly characterized, and only one of which is also expressed after TCR/CD3 stimulation. These results indicate that IL-2 induces a set of genes that is distinct from the TCR/CD3-stimulated "competence" genes. Moreover, the procedures described in this report should have wide applicability in the selective isolation of genes expressed as a result of diverse signaling pathways and

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Abbreviations: IL, interleukin; IL-2R, IL-2 receptor; CHX, cycloheximide; CR, cytokine-responsive; TCR, T-cell antigen receptor.

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should open the way toward the further identification and characterization of cytokine-responsive (CR) genes.

METHODS

Chemicals. Homogeneous recombinant IL-2 was provided by Takeda (Osaka) as a 1.0 mg/ml solution in ammonium acetate buffer (pH 5.0). 4-Thiouridine was obtained from Sigma, and phenylmercury agarose (Affi-Gel 501) was from Bio-Rad. Phorbol 12,13-dibutyrate was obtained from Consolidated Midland (Brewster, NY) and solubilized in dimethyl sulfoxide to yield a stock concentration of 5.0 mg/ml. [³H-methyl]Thymidine (10 Ci/mmol; 1 Ci = 37 GBq) was purchased from ICN, and [5,6-³H]uridine (48 Ci/mmol) was from Amersham.

Cell Culture. Human peripheral blood mononuclear cells were isolated by Ficoll/Hypaque discontinuous centrifugation and cultured at 10⁶ cells per ml in complete medium comprised of RPMI 1640 (GIBCO) supplemented with 10% heat-inactivated (56°C, 30 min) calf serum (Sterile Systems, Logan, UT), 50 µg of gentamycin per ml, 200 µg of L-glutamine per ml, and 50 units of penicillin per ml. T cells were activated by stimulation of the CD3 component of the T-cell receptor complex with an anti-CD3-reactive monoclonal antibody (OKT3, 1:10,000 dilution, Ortho Pharmaceuticals) in the presence or absence of 10 µg of CHX per ml, and DNA synthesis was monitored at 48–52 hr by adding 0.5 µCi of [³H]thymidine to aliquots (200 µl) of cell cultures in 96-well microtiter plates. Cultures were harvested onto glass fiber filters, radioactivity was assayed by liquid scintillation, and [³H]thymidine incorporation was calculated as cpm per 10⁴ cells per hr.

IL-2R-positive T-cell blasts were prepared by stimulation of peripheral blood mononuclear cells with OKT3 for 3 days, after which the cells were washed and replaced in culture for an additional 11 days in the presence of 500 pM IL-2. The cells were subsequently washed and placed in culture in the absence of IL-2 for 36 hr, followed by a 12-hr stimulation with 50 ng of phorbol 12,13-dibutyrate per ml to augment high-affinity IL-2R expression. Cells were washed free of phorbol 12,13-dibutyrate and placed in culture for 12 hr prior to restimulation. Such treatment enabled the generation of a G₀/G₁-synchronized cell population, comprised of >90% T8-positive T lymphocytes (32).

cDNA Library Construction. Human IL-2R-positive T-cell blasts were cultured in the presence of 1 nM IL-2, 10 µg of CHX per ml, 250 µM 4-thiouridine, and 2.5 µCi of [³H]uridine per ml for 2 hr. The cells were then lysed in guanidium thiocyanate and total cellular RNA was isolated as described (33). To purify thiol-labeled transcripts, the total RNA was dissolved in loading buffer consisting of 0.05 M NaOAc (pH 5.5), 0.1% SDS, 0.15 M NaCl, and 4 mM EDTA and heated at 65°C for 5 min. The RNA was then cooled on ice and adsorbed for 10 min at room temperature to a phenylmercury agarose (Affi-Gel 501) column, which had been equilibrated in the RNA loading buffer. Approximately 400 µg of RNA was applied per 1-ml packed column resin. The unbound eluate was collected, reheated, and reapplied to the column. The column was then washed with the RNA loading buffer, followed by loading buffer containing 0.5 M NaCl to remove nonspecifically adhering transcripts. The thiol-labeled RNA was eluted in loading buffer containing 50 mM 2-mercaptoethanol (31). The thiol-selected RNA was precipitated in ethanol and used in the synthesis of *Not* I primer/adaptor-primed cDNA, utilizing the Riboclone cDNA synthesis system (Promega) according to the manufacturer's instructions. After addition of *Eco*RI adaptors (Promega), *Not* I digestion, and size selection for fragments >500 bp, the cDNA was ligated directionally into an *Eco*RI- and *Not* I-digested pBluescript II SK+ plasmid vector (Stratagene), followed by

transformation into Epicurian *Escherichia coli* XL1-blue competent cells (Stratagene).

Colony Screening. Single-stranded ³²P-labeled cDNA probes were prepared from poly(A)⁺ RNA isolated from human T-cell blasts stimulated for 2 hr with medium (unstimulated probe) or 1 nM IL-2 and 10 µg of CHX per ml (stimulated probe). Total cellular RNA was prepared as described (33), and poly(A)⁺ RNA was isolated by three passages over an oligo(dT)-cellulose column (5 Prime → 3 Prime, Inc.). First-strand cDNA synthesis was performed with an oligo(dT)_{12–18} primer (United States Biochemical), using the Riboclone cDNA synthesis system (Promega) according to the manufacturer's instructions, with the exception of dCTP at a final concentration of 35 µM and the addition of 2.5 mCi of [³²P]dCTP per ml. Hybridization was carried out for 72–96 hr at 42°C in 50% formamide, with a final probe concentration of ≈2 × 10⁶ cpm/ml (34). Subsequent to hybridization, filters were washed repeatedly at 62°C in 0.1 × SSC (1 × SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) and 0.1% SDS and placed on film (Kodak XAR-5) with DuPont Cronex intensifying screens overnight at –70°C. Colonies that exhibited differential hybridization to the stimulated and unstimulated probes after three independent screens were isolated for further analysis.

Northern Blot Analysis. Total cellular RNA was isolated by the guanidine thiocyanate method (9) and denatured in glyoxal and dimethyl sulfoxide, followed by fractionation on a 1% agarose gel in 0.01 M NaH₂PO₄ with 0.5 µg of ethidium bromide per ml (35). To estimate sizes of RNA transcripts, a 0.24- to 9.5-kb RNA ladder (Bethesda Research Laboratories) was run alongside the cellular RNA samples. After visualization under UV light, the RNA was transferred to nitrocellulose by capillary transfer in 10 × SSC. Plasmids were purified from the clones of interest, and the *Not* I- and *Eco*RI-excised inserts were ³²P-labeled with random primers. Hybridization was carried out in 50% formamide at 42°C for 48–72 hr, followed by repeated washes in 0.1 × SSC/0.1% SDS at 56–62°C (35). Filters were exposed to Kodak XAR-5 film with DuPont Cronex intensifying screens, and specific bands were quantitated with an EC densitometer (E-C Apparatus). Filters were normalized for RNA loading by hybridization with a 1.2-kb fragment of the cDNA encoding the *cdc*-like kinase, *clk* (36).

Sequence Analysis. Plasmids were isolated from the clones of interest (37) and vector primers were used to sequence the termini of the cDNA inserts, employing the Applied Biosystems model 373A DNA sequencer. Further sequence analysis was performed using a combination of exonuclease III-generated nested deletions (38) and custom-designed oligonucleotide primers. Searches of the GenBank and EMBL data bases were performed with the FASTA program as described (39).

RESULTS

Thiol-Labeled RNA Purification and cDNA Library Construction. To construct the cDNA library, total cellular RNA was fractionated by passage over a phenylmercury agarose column, such that thiol-labeled transcripts were covalently bound to the column, whereas unlabeled RNA was collected in the column flow-through. Nonspecifically bound RNA was removed from the column by low (0.15 M NaCl) and high (0.5 M NaCl) salt washes, and the thiol-labeled RNA was subsequently eluted in the presence of 50 mM 2-mercaptoethanol (30, 31). In several such experiments, >90% of the total RNA was recovered in the flow-through and nonspecifically bound column fractions, whereas the labeled RNA was specifically eluted with 2-mercaptoethanol. Thus, this purification resulted in an ≈10-fold enrichment for newly synthesized [³H]uridine-labeled RNA, calculated by the increased specific activity of the recovered ³H-labeled transcripts (Fig.

1A). The purification scheme was specific for thiol-labeled transcripts, because when the 4-thiouridine was omitted from the procedure, only 0.2% of the total [^3H]RNA was recovered in the 2-mercaptoethanol eluate. When the nonselected total RNA was compared with the nonspecifically bound RNA and thiol-selected RNA by Northern hybridization using a probe encoding the known IL-2-induced immediate-early gene *c-myc* (26), a marked increase in the relative abundance of *c-myc* was observed in the thiol-selected RNA (Fig. 1B). Therefore, this thiol-selected RNA, which constituted 7.5% of the total cellular RNA, was used in the synthesis of an oligo(dT)-primed cDNA library of $\approx 10^4$ clones in the plasmid vector pBluescript II SK $^+$.

Northern Blot Analysis of Putative Clones. Differential colony screening of $\approx 10\%$ of the library yielded 18 putative IL-2-induced genes, as assessed by greater hybridization to single-stranded ^{32}P -labeled cDNA probes generated from 2-hr IL-2- and CHX-treated versus unstimulated human IL-2R-positive T-cell RNA. Inasmuch as CHX was included in the library and probe preparation, it was essential to verify that the differential expression of putative clones observed upon colony screening was not due solely to the effects of this drug. In addition, determination of the sizes and patterns of induction of the RNA transcripts was necessary to enable estimation of the redundancy of the clones. Therefore, Northern blot analysis was performed with RNA isolated from human IL-2R-positive T-cell blasts stimulated for 2 hr with either 10 μg of CHX per ml or 1 nM IL-2 alone or a combination of the two agents. Hybridization of the RNA with probes generated from the inserts of each of the 18 putative clones resulted in the identification of 4 clones that

were solely CHX-induced. For the remaining 14 clones, the induction by the combination of IL-2 and CHX could not be accounted for by the effects of CHX alone. Based upon the patterns of induction and approximate sizes of the RNA transcripts, 8 readily distinguishable and apparently unique IL-2-induced genes were discerned among these 14. These IL-2-induced genes were termed CR 1–8. The IL-2 induction of these clones could be readily detected after autoradiographic exposure of the Northern filters for periods ranging from 4 hr (clone CR-8) to overnight (clone CR-2). As shown in Fig. 2, 3 of the genes (CR-1, -3, -5) were induced by only IL-2 alone, whereas 5 of the genes (CR-2, -4, -6, -7, -8) were induced by CHX and IL-2. Also, it is noteworthy that the combination of IL-2 and CHX resulted in a marked synergistic induction in most instances.

Sequences of IL-2-Induced Genes. To verify the redundancy of the clones as estimated from Northern analysis, and to determine the identities of the genes, the cDNA clones were subjected to sequence analysis. The combination of sequence and Northern analysis revealed that the 14 putative IL-2-induced clones consisted of eight unique genes, three of which (CR-1, -6, -8) were isolated three times each. Searches of the GenBank and EMBL data bases (39) with the sequences of the eight clones enabled the identification of two clones. CR-4 is a recently identified DNA-binding protein, SATB1, which binds nuclear matrix-associated DNA (40), and CR-7 is *pim-1*, a previously characterized IL-2-induced gene (26, 41) that encodes a 33-kDa cytoplasmic kinase (42). The sequences of the other six clones did not show complete identity with any genes in the current data bases (Table 1). The sequence of the 0.8-kb CR-6 cDNA showed $\approx 65\%$ homology at the DNA level, and 40% identity to the predicted amino acid sequences of three known genes, the human and hamster GADD45 genes, and the mouse MyD118 gene (data not shown). The 1.3-kb GADD45 gene was initially isolated as a UV-irradiation-inducible gene in Chinese hamster ovary cells (43), and the human homologue, which is 91% identical to the hamster gene, was subsequently cloned from a human fibroblast library (44). The 1.3-kb mouse MyD118 clone was isolated by virtue of its induction by IL-6 in a murine myeloid cell line (45, 46). The deduced amino acid sequences of these proteins, which predict products of 17–18 kDa, do not exhibit

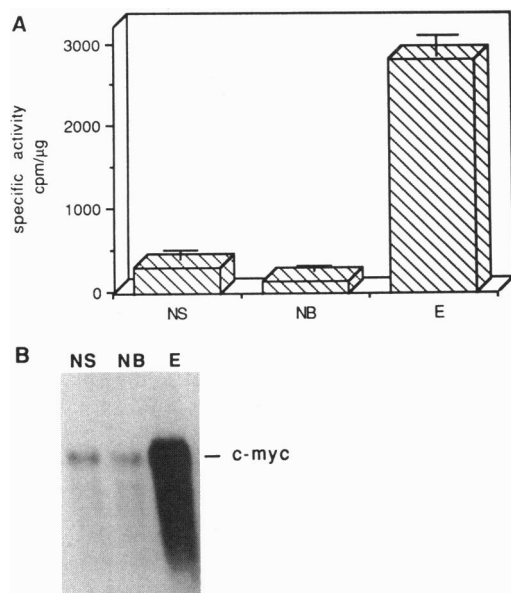


FIG. 1. Purification of thiol-labeled RNA. (A) Total cellular RNA was isolated from 2-hr IL-2- and CHX-stimulated human peripheral blood T cells, and thiol- and [^3H]uridine labeled transcripts were purified by passage over a phenylmercury agarose column (see text). Column fractions were collected that contained the unbound flow-through and the nonspecifically bound RNA (NB) and the thiol-labeled RNA specifically eluted with 2-mercaptoethanol (E). The specific activity of the RNA, in terms of cpm of [^3H]uridine per μg of RNA, was determined for the nonselected total RNA prior to separation (NS) as well as for each of the column fractions. Data are represented in terms of the mean \pm SEM and are representative of three separate experiments. (B) RNA samples separated as in A, comprising the total nonselected RNA (NS), the nonspecifically bound RNA (NB), and the specifically bound and eluted RNA (E), were analyzed by Northern blot hybridization with a *c-myc* cDNA probe (25).

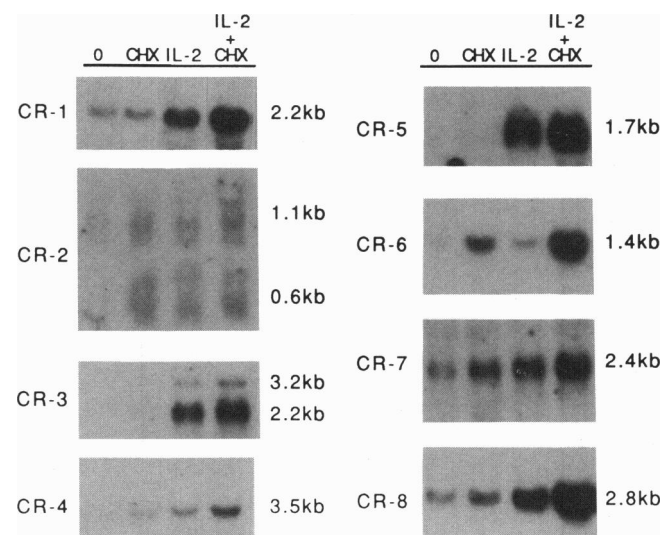


FIG. 2. Northern blot analysis of expression of putative IL-2-induced genes. G_0/G_1 -synchronized human T cells were stimulated for 2 hr with 10 μg of CHX per ml or 1 nM IL-2 alone or a combination of the two agents. Following electrophoresis of 15 μg of total RNA and transfer to nitrocellulose, filters were probed with ^{32}P -labeled cDNA inserts of putative IL-2-induced clones.

Table 1. Characterization of IL-2-induced genes

Clone	Insert, kb	RNA, kb	IL-2 induction*	Identity†
CR-1	1.6	2.2	24	?
CR-2	1.1	0.6, 1.1	7	?
CR-3	1.8	2.2, 3.2	22	?
CR-4	0.8	3.5	6	SATB1
CR-5	1.3	1.7	>50	?
CR-6	0.8	1.4	5	?
CR-7	0.7	2.4	17	<i>pim-1</i>
CR-8	1.3	2.8	7	?

*Maximum fold induction (relative to unstimulated cells) of steady-state RNA levels in human T cells during 8 hr of IL-2 stimulation as determined from densitometric scans of Northern blots (Fig. 3).

†Identity of clone based upon searches of GenBank and EMBL data bases (39).

significant homologies to other proteins in the current data bases, and their functions are not yet known (44, 46).

Kinetic Analysis of IL-2-Induced Gene Expression. The kinetics of induction of previously characterized IL-2-responsive genes have been found to range from those such as *c-fos*, which are rapidly and transiently induced within minutes of IL-2 stimulation (26), to those that remain at elevated levels through G₁ to S phase entry (47). The temporal expression of the CR genes was determined by Northern blot analysis, using RNA isolated from human IL-2R-positive T-cell blasts after IL-2 stimulation in the presence or absence of CHX (Fig. 3). Two of the genes, CR-1 and CR-4, exhibited rapid induction, reaching peak levels within 1–4 hr of IL-2 stimulation and returning to basal levels after 8 hr, whereas the other six clones remained at elevated levels for at least 8 hr in

the presence of continuous IL-2 treatment. The magnitude of IL-2 induction of steady-state RNA levels of the clones ranged from an \approx 5-fold elevation of clone CR-6 to a >50-fold stimulation of clone CR-5 during the interval examined (Table 1). Several of the clones were superinduced by CHX, with an increase observed in the magnitude and duration of the IL-2 response. The CHX effect resembles that previously described for a number of immediate-early genes (27–29).

Responsiveness to TCR Stimulation. To determine whether the IL-2-induced genes were also sensitive to TCR/CD3 triggering, Northern blot analysis was performed with RNA isolated from human peripheral blood mononuclear cells stimulated with an antibody (OKT3) specific to the CD3 component of the T-cell antigen receptor complex. To verify that the cells were significantly activated via CD3, aliquots of the cells were left in culture for 48 hr, after which cell cycle progression was monitored by [³H]thymidine incorporation. Such analysis demonstrated that the anti-CD3 stimulation was sufficient to induce DNA replication (stimulation index = 69). For Northern blot analysis, RNA was isolated after 2 hr of anti-CD3 treatment, so that it was possible to identify those genes that were induced by TCR stimulation in the absence of IL-2 effects (22). Even after autoradiographic exposure of the Northern blots for several days, transcripts of the majority of CR genes were undetectable, and only one of these genes (CR-4) was found to be induced by TCR activation (data not shown). Thus, the induction of seven of the CR genes was unique to the IL-2 signaling pathway.

DISCUSSION

By utilizing a differential hybridization approach that incorporates CHX superinduction of immediate-early gene expression (27–29) in combination with a thiol-labeling procedure to select for newly synthesized RNA (30, 31), we have cloned eight IL-2-induced genes. The differential cloning methods employed in this study are derived from those described by Cochran *et al.* (27), who identified cell cycle competence genes induced by platelet-derived growth factor (PDGF) in murine embryonic (3T3) fibroblasts. Subsequently, their approach was confirmed and extended by Lau and Nathans (28) and Almendral *et al.* (29), who utilized CHX superinduction of serum-induced 3T3 cell gene expression to further characterize competence genes. Our results with IL-2-induced genes are in accordance with these earlier observations, as CHX treatment considerably augmented the IL-2 stimulation of the clones described here. In addition, the purification of newly synthesized IL-2-induced transcripts afforded by the thiol-labeling procedure employed in this study (31) enabled the generation of a cDNA library from <10% of the total cellular RNA and thus further heightened the efficacy of the cloning procedure.

With regard to the previously described competence genes detected in serum- and PDGF-induced fibroblasts (27–29, 48) and lectin- and phorbol ester-treated T cells (25), it is notable that of the eight IL-2-induced G₁ progression genes reported here, only one (CR-4) appears to also be significantly induced during the TCR-mediated competence phase of the cell cycle. This result suggests that, although several genes such as *c-fos*, *c-myc*, and *c-raf-1* are induced during the initial G₀–G₁ and subsequent G₁–S phase transitions (22, 23, 26), the expression of a number of IL-2-stimulated genes is unique to the latter event. In addition, the immediate-early genes reported here appear to define a class distinct from the IL-2-induced genes isolated by Sabath *et al.* (47). These investigators also utilized a differential screening procedure, but isolated genes expressed later, at the G₁/S phase boundary, in a murine T-helper clone that was stimulated with IL-2 for 20 hr without protein synthesis inhibitors. Of the 21 cDNAs identified, 14 were found to be already known genes, including various metabolic enzymes and cytoskeletal pro-

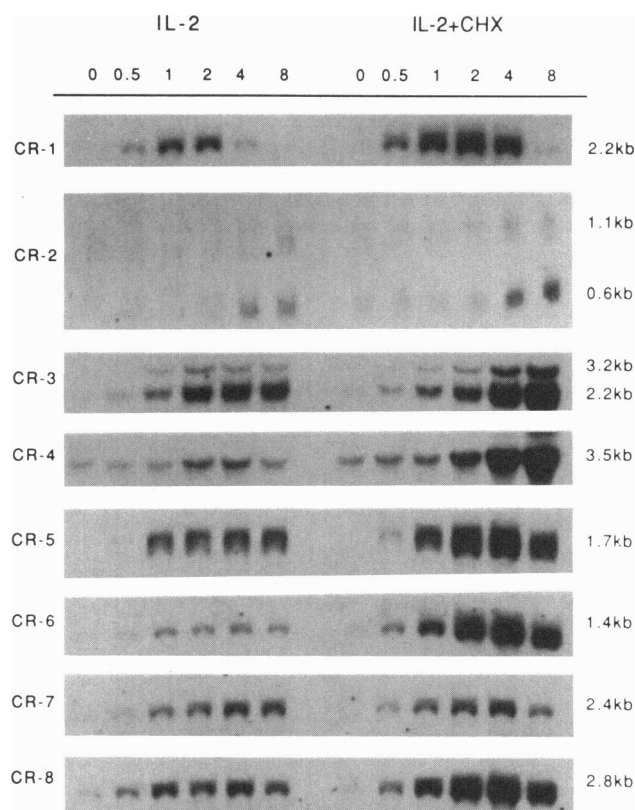


FIG. 3. Kinetics of IL-2-induced gene expression. Northern blots were prepared with 15 μ g of total RNA isolated from G₀/G₁-synchronized human T cells stimulated for the indicated times, in hours, with 1 nM IL-2 or IL-2 plus 10 μ g of CHX per ml. Filters were probed with the cDNA inserts of the IL-2-induced clones.

teins, whereas 7 represented previously unrecognized sequences. In addition, the expression of 3 of the clones was actually inhibited by CHX, whereas the remainder were insensitive to this agent. This pattern of regulation contrasts markedly with the CHX superinduction observed with the immediate-early IL-2-induced genes described here. Moreover, these observations indicate that IL-2 stimulates a complex program of gene expression, ranging from those genes induced very early in G₁ through those subsequently expressed at the G₁/S phase transition.

It is anticipated that the immediate-early genes expressed in response to IL-2 stimulation will eventually be classified into distinct families of CR genes, based upon sensitivity of induction by additional cytokines. Recently, the 64-kDa and 75-kDa components of the IL-2R have been found to be members of a cytokine receptor superfamily, which includes the receptors for erythropoietin, growth hormone, prolactin, granulocyte and granulocyte-macrophage colony-stimulating factor, IL-3, -4, -6, and -7, as well as the 130-kDa signal-transducing component of the IL-6 receptor (49–54). Although the criteria utilized in defining membership in this superfamily have primarily focused upon sequence homologies within the ligand-binding domains of the receptors, there may also be similarities within the intracellular regions of the receptors that are coupled to cytoplasmic signaling pathways. Thus, it is noteworthy that mutational analyses of the cytoplasmic domains of the erythropoietin receptor (55, 56), the IL-2R p75 (57), and the 130-kDa signal-transducing component of the IL-6 receptor (54) indicate that the region proximal to the membrane is required for efficient signal transduction and that sequence homologies have been found among members of the receptor family within this functionally important region (54). In addition, the finding that the IL-2R p75 (57) and erythropoietin receptor (58) can confer responsiveness to their respective ligands in IL-3-dependent cells is suggestive of commonalities in the signaling mechanisms activated by these receptors. Thus, in light of the sequence homologies that define the cytokine receptor superfamily, it will be of interest to determine to what extent the family can be further subdivided on the basis of the composition and structure of the receptor cytoplasmic domains, the biochemical signaling pathways triggered by the receptors, and, finally, the patterns of induction of immediate-early genes affected therein. Accordingly, an approach is now available to extend the analyses of the various cytokine receptors to encompass the characterization of their target CR genes.

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